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WO 2004/035784 A3

(54) Title: SOLUBLE RNA POLYMERASE PROTEIN AND METHODS FOR THE USE THEREOF

(57) Abstract: A polymerase protein originating from a eukaryotic cell and involved in the RNA silencing pathway is for the first time provided in a purified soluble form that possesses a detectable RNA polymerization activity. This polymerase is useful in methods and kits for *in vitro* RNA synthesis. A polymerase of the invention copies ssRNA templates to produce two types of reaction products: short and long RNA copies. It can also copy ssDNA templates. The polymerization does not require a primer for the initiation of RNA synthesis, although RNA synthesis can be also initiated in the presence of a primer. In addition to standard nucleotides polymerase of this invention also accepts a number of modified nucleotides. The polymerase is useful in many downstream applications such as production of labeled RNA probes or generation of trigger RNA molecules to induce RNA interference effects in living cells or suitable *in vitro* systems.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/FI 03/00776

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N9/12 C12N15/11 C12Q1/68 C12N15/10 C12P19/34

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	MAKEYEV, EUGENE V. ET AL: "Cellular RNA-Dependent RNA Polymerase Involved in Posttranscriptional Gene Silencing Has Two Distinct Activity Modes" MOLECULAR CELL, vol. 10, December 2002 (2002-12), pages 1417-1427, XP002278527 the whole document	1-41
X	DATABASE GENBANK [Online] XP002278532 Database accession no. CAC10121 100% identity in 1026/1402 a.a. of SEQ ID No 4, 100% identity in 1402/1402 a.a. of SEQ ID No 2. abstract	12-17

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 April 2004	20. 07 2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Brouwer, W

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/FI 03/00776

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category°	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	-& WO 00/50581 A (UNIVER ROMA) 31 August 2000 (2000-08-31) page 8, line 5 - line 22 abstract	1-41
X	SCHIEBEL, WINFRIED ET AL: "RNA-directed RNA Polymerase from Tomato Leaves" THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 263, no. 16, 1993, pages 11851-11857, XP002278528 the whole document	1-41
X	US 2001/023067 A1 (SCHIEBEL WINFRIED ET AL) 20 September 2001 (2001-09-20) see page 2, left column paragraph [0013]	1-41
A	CATALANOTTO, CATERINA ET AL: "Involvement of small RNAs and role of the qde genes in the gene silencing pathway in Neurospora" GENES & DEVELOPMENT, vol. 16, no. 7, 1 April 2002 (2002-04-01), pages 790-795, XP002278529 the whole document	1-41
A	COGONI, CARLO ET AL: "Gene silencing in Neurospora crassa requires a protein homologous to RNA-dependent RNA polymerase" NATURE, vol. 399, 13 May 1999 (1999-05-13), pages 166-169, XP002278530 the whole document	1-41
A	COGONI, CARLO ET AL: "Isolation of quelling-defective (qde) mutants impaired in posttranscriptional transgene-induced gene silencing in Neurospora crassa" PROC. NATL. ACAD. SCI., vol. 94, September 1997 (1997-09), pages 10233-10238, XP002278531 the whole document	1-41

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 03/00776

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-9 and 20-41 (all partially)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-12 and 14-41

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-9 and 20-41 (all partially)

Present claims 1-9 and 20-41 relate to the use of a product which is defined by reference to a desirable characteristic or property, namely the capability of producing short complementary RNA copies of a template. The claims cover all products, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such products. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the use of RNA polymerase QDE-1 or the enzymatically active C-terminal fragment of QDE-1.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-12 and 14-41

   directed to an isolated soluble RNA dependent RNA polymerase and its use.

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2. claim: 13

   directed to a mutated RNA polymerase lacking polymerase activity.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/FI 03/00776

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0050581 A	31-08-2000	IT RM990117 A1	22-08-2000
		AU 3189700 A	14-09-2000
		CA 2362203 A1	31-08-2000
		EP 1155122 A1	21-11-2001
		WO 0050581 A2	31-08-2000
US 2001023067 A1	20-09-2001	US 6218142 B1	17-04-2001